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Premotor cortex of rhesus monkeys: set-related activity during two conditional motor tasks

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Summary. We compared set-related premotor cortex activity in two conditional motor tasks. In both tasks, a rhesus monkey moved its forelimb to one of two possible targets on the basis of visuospatial instruction stimuli. One target was located to the left of the limb's starting position, the other to the right. In the *directional task*, a white light situated within the target provided the instruction. In the *arbitrary task*, colored instruction stimuli equidistant from the targets established an arbitrary relationship between stimulus and response. One hypothesis about set-related premotor cortex activity is that it contributes to the preparation for limb movement on the basis of sensory instruction stimuli. If set-related activity differed profoundly in the arbitrary and directional tasks, then that hypothesis would be untenable. Out of 403 task-related premotor cortex neurons in two monkeys, 130 neurons showed set-related activity, and we studied 118 cells in detail. The vast majority (81%) of these 118 neurons showed no significant difference between the two tasks in set-related activity. When set-related activity did differ, the greatest activity usually occurred after arbitrary instructions; the opposite being the case for only 5% of our sample. Differences in activity during the two tasks, even when statistically significant, were generally small. The present results accord with the hypothesis that set-related premotor cortex activity reflects aspects of motor preparation.

Key words: Premotor cortex – Frontal lobe – Area 6 – Motor preparation – Motor set

Introduction

The primate premotor cortex, a distinct cortical field (or set of fields) within the agranular frontal cortex (Muakkassa and Strick 1979; Humphrey 1979; Wiesendanger 1981; Halsband and Passingham 1982; Brinkman and Porter 1983; Wise 1984, 1985a; Goldberg 1985; Freund and Hummelsheim 1985; Matelli et al. 1985, 1986), has been the subject of a number of recent ablation studies that implicate it in the control of conditional motor behavior. Petrides (1982, 1985a, b, c, 1986), Halsband and Passingham (1982, 1985) and Passingham (1985, 1986) have shown that monkeys with damage to the premotor cortex perform extremely poorly, often at levels no better than chance, on learning or relearning certain conditional motor tasks[1] (see also Goldman and Rosvold 1970; Goldman et al. 1971). Halsband and Passingham (1982, 1985) found that ablation of the premotor cortex leads to severe deficits when a color cue instructs the monkeys to make one of two movements (either pull or twist a manipulandum). However, when a similar cue determines which of two objects will, when displaced, lead to a reward, monkeys with the same or similar lesions can perform as well as intact animals (Passingham 1985). Further, when a manipulandum's color indicates whether it should be twisted or pulled, the monkeys perform well (Passingham 1986). Petrides (1985c) has similarly shown that ablation of part of the premotor cortex, termed the postarcuate area (or the superior premotor area of Matelli et al. 1986), leads to deficits on certain conditional tasks. Conditional tasks spe-

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1 A conditional task is one in which two stimulus-response associations determine the correct behavior on a test: if one stimulus is presented then a certain behavior is correct; if a second stimulus occurs then a different behavior is correct

cifically affected include those in which the behaviors differ kinesthetically, i.e., are different movements. Monkeys with postarcuate ablations perform poorly when stimuli indicate whether to perform a behavior or withhold performance, but perform well when a visual cue indicates whether a reward is located in a lit vs. an unlit box. It appears that monkeys with premotor cortex ablations can learn tasks involving associations of arbitrary stimuli with objects, or objects (such as colored manipulanda) with movements, but not associations of arbitrary stimuli with movements when animals must choose among a number of arbitrary stimulus-response relationships. This dissociation provides the strongest evidence that the contribution of the premotor cortex to behavior is inexplicable solely in terms of traditional motor control concepts (cf. Freund and Hummelsheim 1985) and suggests that the premotor cortex is specialized for control of conditional visuomotor behavior. Indeed, depending upon the definition of a conditional motor task, both Petrides (1986) and Passingham (1986) have argued that premotor cortex (or periarcuate) lesions specifically disrupt performance of such tasks. Accordingly, premotor cortex neuronal activity during a conditional motor task is of considerable interest.

Neurophysiological studies to date, with the exception of that of Godschalk et al. (1985), have studied premotor cortex neuronal activity during the performance of tasks that were conditional by the broad definition of Footnote 1, but not by more restrictive definitions. In most studies there were clear directional relationships between cues and behavior. For example, in some studies monkeys depressed a target key that contained (Weinrich and Wise 1982; Wise and Mauritz 1985; Mauritz and Wise 1986) or was near (Vaadia et al. 1986) a sensory cue that designated it, among a number of potential targets, to be the target of the next limb movement. Movements to grasp a reward (Godschalk et al. 1981, 1983) or a manipulandum (Brinkman and Porter 1983) are of a similarly directional nature. Other neurophysiological studies involved sensory cues that, while physically abstracted from the target of the limb movement, nevertheless had a spatial component comparable to the movement that they instructed (Kubota and Hamada 1978; Weinrich et al. 1984). While, in a sense, these might be considered conditional motor tasks, and are treated as such in this report (see Footnote 1), the clear directional coupling between stimulus and response inherent in most previous neurophysiological studies differs from the usual conditional learning situation in which "arbitrary responses . . . bear no relation to the stimuli" (Petrides 1986, p. 2054).

One published neurophysiological study did involve a more usual conditional motor task. Godschalk et al. (1985) compared neuronal activity in premotor cortex before and during movements instructed by direct vision of the target with those instructed by an arbitrarily selected pattern of visual signals. Their results indicated that premotor cortex activity during a delay period between the stimulus and movement did not substantially differ following direct vision and arbitrary instructions. However, the number of cells studied in both conditions was relatively small (13) and Godschalk et al. recorded mainly from a more laterally situated part of the premotor cortex than we have previously studied in detail, a region argued by some to constitute a separate motor representation (Matelli et al. 1986). In view of the paucity of information on the superior aspect of premotor cortex and its activity during a clearly conditional motor task, we compared set-related activity of premotor cortex neurons during two tasks: in one, instruction stimuli were directly incorporated into a target and therefore bore a clear spatial relationship to the response, whereas, in the other, such stimuli were arbitrary in that they contained no inherent directional information.

Material and methods

Behavioral paradigm

Two male rhesus monkeys (*Macaca mulatta*), 5 kg and 6 kg, were used in the present experiments. They were cared for in accordance with the *Guiding Principles in the Care and Use of Laboratory Animals* of the American Physiological Society. Each monkey was seated in a primate chair and operantly conditioned to touch one of three metal touch pads with its left arm in response to visual cues. Its right arm was loosely restrained with a metal bar around the upper arm.

The three rectangular touch pads, 6.5 cm wide by 5.8 cm high, were located on a panel at arm's length in front of the monkey and separated by 10 cm horizontally. Three incandescent lamps, white, blue, and yellow, were aligned straight from bottom to top, in that order, above the central touch pad. Figure 1 shows two of these three lamps, the blue and the yellow, in each of the four schematic diagrams of the panel. The white lamp, which was immediately above the central touch pad, is not shown. One white lamp each was also inserted into a hole within the left and right touch pads. The clear casing of the lamp filled the hole, 1 cm in diameter, and was centered 5 cm from the bottom of the pad. All five lamps had identical size and shape. In addition to the incandescent lamps, red light emitting diodes (LEDs) were located below each of the three touch pads.

After illumination of the central white lamp and LED, the monkey initiated a trial by touching the center pad with its left hand. After a 0.5 s or 1 s period, one of the following four lamps, the central blue, the central yellow, the left white or the right white lamp, was illuminated as an *instruction stimulus* (IS), and the central lights were turned off. The target for a trial was the *left* touch pad if the IS was either the left white or the central yellow lamp and was the *right* touch pad if the IS was either the right

Table 1. Reaction time (RT) and movement time (MT) for conditioned forelimb movements

Type of IS	RT Mean ± S.D.[a]	MT Mean ± S.D.
Monkey 1 (nondirectional TS)[c]		
Arbitrary left	371 ± 35[b]	207 ± 22
Directional left	379 ± 15	203 ± 21
Arbitrary right	340 ± 16	204 ± 19
Directional right	337 ± 57	197 ± 16
Monkey 1 (directional TS)		
Arbitrary left	366 ± 14	176 ± 9
Directional left	390 ± 26	188 ± 15
Arbitrary right	360 ± 17	183 ± 13
Directional right	401 ± 43	174 ± 25
Monkey 2 (nondirectional TS)		
Arbitrary left	355 ± 18	105 ± 15
Directional left	360 ± 25	106 ± 11
Arbitrary right	307 ± 27	126 ± 14
Directional right	313 ± 27	130 ± 13

[a] Standard deviation
[b] Means in ms
[c] A nondirectional TS consisted of the illumination of the LEDs beneath both targets. A directional TS involved the illumination of just the LED beneath the target. Each trial involved the presentation of an IS

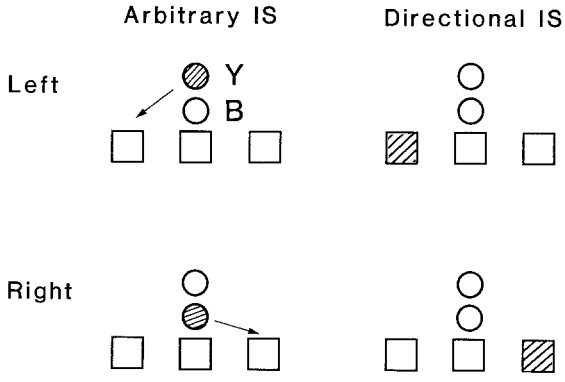

Fig. 1. Four schematic diagrams of the panel showing the four possible instruction stimuli. Hatching indicates lamp illumination. Arrows indicate the associated direction of forelimb movement for the colored stimuli. Illumination of the yellow (Y) or blue (B) lamp was used for an arbitrary instruction stimulus, whereas illumination of left or right lamp served as a directional instruction stimulus

white or the central blue lamp (Fig. 1). The *directional* task involved the illumination of either the left or right white light within the target. Presentation one of the colored instruction stimuli constituted the *arbitrary* task, so termed because the central blue and yellow lights were located the same distance from the left and right touch pads and contained no inherent directional information.

After presentation of a randomly selected IS, equally weighted among the four possibilities, the monkey maintained contact with the central touch pad for a randomized *instructed delay period* (one of the set ranging from 1.52 s to 2.36 s in 0.12 s steps). After the delay period both the left and the right LEDs were simultaneously illuminated to serve as a *nondirectional* trigger signal (TS). The monkey had to touch the target within 600 ms of the TS onset to get a drop (0.1 ml) of juice as a reward. After the monkey received a reward, the central lamp and LED were illuminated to indicate that a new trial could be initiated. No intertrial period was imposed.

In most of the trials, the IS remained on for the entire delay period and until the monkey contacted either the left or right touch pad ("IS-on" mode). When a single cell's activity was recorded for 80 to 120 trials, we examined it when the presentation of the IS and/or the TS was altered. The IS was either eliminated at the same time the TS was presented ("IS-blank" mode), or the IS was removed after 1 s in the first monkey and 0.5 s in the second monkey ("IS-off" mode). The monkeys could also be presented with a directional TS, the illumination only of the LED beneath the target. Regardless of the type of TS, every trial included the presentation of an IS. A selection of these task variations was presented for blocks of 80 to 120 trials, after which it was possible in 19% of the cases to monitor the unit in the IS-on mode with a nondirectional TS, i.e., the original situation, for an additional 80 to 120 trials.

Recording methods

After about 8 weeks, when the monkeys had been trained to perform the task reliably, they were anesthetized with sodium pentobarbital (30 mg/kg) following induction with ketamine hydrochloride (8 mg/kg), and a stainless steel recording chamber (27 mm × 27 mm) was implanted over the right hemisphere. In the same aseptic surgical procedure, head-restraint bolts were attached to the skull. Intramuscular injections of morphine (0.5 mg/kg, twice per day for three days) and antibiotics were delivered to prevent postsurgical pain and infection. After a recovery period of at least three days, glass-insulated platinum-iridium microelectrodes (0.8–1.5 MΩ at 1 kHz) were inserted through the dura mater into the cortex while the monkey performed the tasks. Single units were isolated, their activity converted into digital data, and those data were stored with the behavioral data for subsequent analysis (Arrington 1986).

Muscle activity was monitored from extensor carpi radialis, flexor carpi ulnaris, biceps brachii, triceps brachii, deltoid, trapezius, supraspinatus, infraspinatus, pectoralis, rhomboid, thoracic and lumbar paravertebral muscles, gluteus maximus, quadriceps, anterior tibialis, and gastrocnemius with surface electrodes. All of the muscles were recorded on both sides of the body both before implantation of the recording chamber and near the completion of single-unit data collection in each monkey. Recorded EMGs were transformed into pulse-replica data by a level discriminator and recorded in the same manner as the single-unit data.

Single-unit data analysis

Single units were studied if they showed noticeable discharge frequency changes during task performance. Neuronal activity was defined as set-related if it showed a statistically significant sustained increase or decrease in discharge rate during the instructed delay period (from 0.5 s to 1.5 s after IS presentation), compared with the discharge rate during the 1 s period from the initiation of the trial to the presentation of the IS (Mann-Whitney U-Test at the 5% significance level). Set-related neurons were not studied unless at least 15 rewarded trials were recorded for each instruction stimulus.

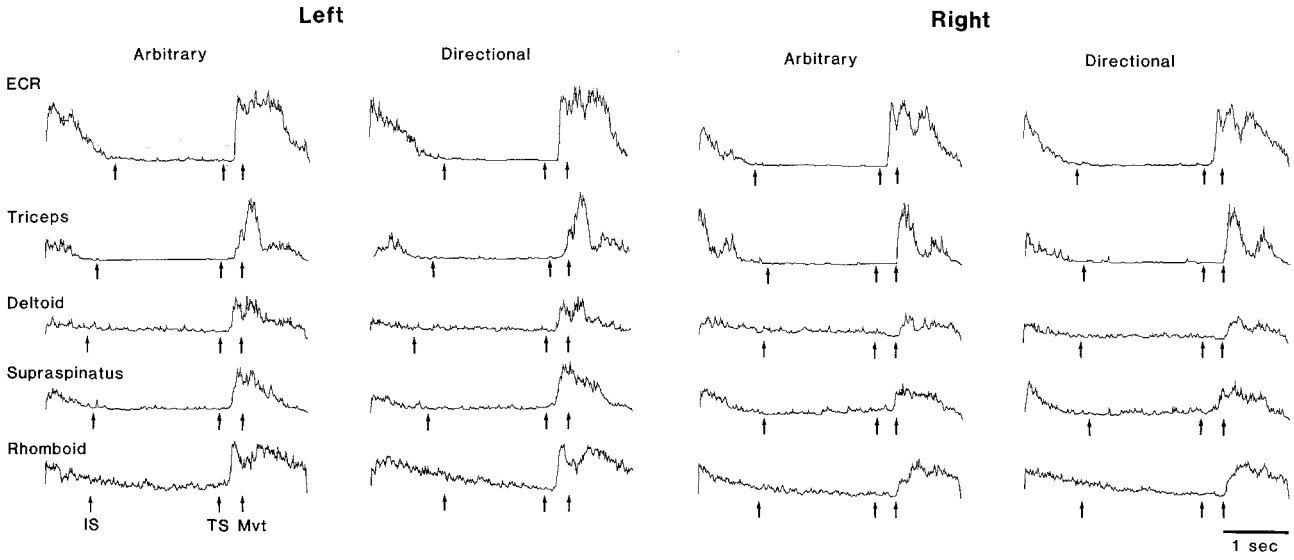

Fig. 2. EMG activity from selected left forelimb muscles in the second monkey. The data are shown in the form of reciprocal interval plots of pulse-replica EMG records (see Material and methods). For each display, EMG activity from 20 trials with comparable instructed delay periods was aligned on the movement onset (Mvt). In each of the four traces in each row, the first arrow indicates the average onset of the instruction stimulus (IS), and the second and the third arrows represent the average onset of the trigger signal (TS) and the movement onset (Mvt), respectively. Activity to the far left of the IS reflects the return of the monkey's limb from the left or right touch pad. Abbreviation: ECR, extensor carpi radialis

Table 2. Classification of neuronal activity patterns

	Anticipatory[a]	Signal	Set	Movement	Total[b]
Monkey 1	27[c]	63	59	114	159
Monkey 2	25	36	71	67	152
Total	52	99	130	181	311
Percentage	17%	32%	42%	58%	

[a] *Abbreviations*: Anticipatory, anticipatory neuron; Signal, signal-related neuron; Set, set-related neuron; and Movement, movement-related neuron

[b] The total number of neurons for each monkey is smaller than the sum of those in each row because some neurons showed combinations of activity patterns

[c] Number of neurons

Table 3. Numbers of set-related neurons grouped according to a comparison of activity in the arbitrary (A) and directional (D) tasks during the instructed delay period, from 500 ms to 1500 ms after IS presentation

	A = D[a]	A > D[b]	A < D[c]
Increased activity	96	16	6
Bidirectional	72	11	6
Directional	24	5	0
Decreased activity	12	0	0
Activity-pattern			
1. Tonic	70	10	5
2. Incremental	6	0	1
3. Decremental	8	1	0
4. Early	6	4	0
5. Late	6	1	0
6. Late, inconsistent[d]	17	0	0

[a] Neurons without statistically significant differences in set-related activity between the arbitrary and directional tasks

[b] Neurons with greater activity in the arbitrary conditional task ($p < 0.05$, Mann-Whitney U-Test)

[c] Neurons with greater activity in the directional conditional task ($p < 0.05$, Mann-Whitney U-Test)

[d] This activity pattern is included as set-related in this table only, and only in its lower part. All these cells had increased activity near the end of the delay period

Histology

After collection of the single-unit data was completed, electrolytic marking lesions were produced by passing 20 μA of direct cathodal current through microelectrodes for 10 s to 15 s. At that time, an incision was made in the dura under aseptic conditions, a 1 μl Hamilton syringe inserted into the cortex at a known coordinate, and 0.1 μl of an axoplasmically transported tracer injected into the cortex. One week later, the monkeys were deeply anesthetized with an overdose of pentobarbital (50 mg/kg) and perfused through the heart with normal saline followed by a fixative containing 3% formaldehyde (w/v). After marking the location of the recording chamber by five pins at known electrode coordinates, the brain was removed from the skull and photographed. Then it was sectioned at 30 μm in the frontal plane on a freezing microtome and stained with thionin. The location of the recording sites were reconstructed by reference to the electrolytic microlesions and the pin holes.

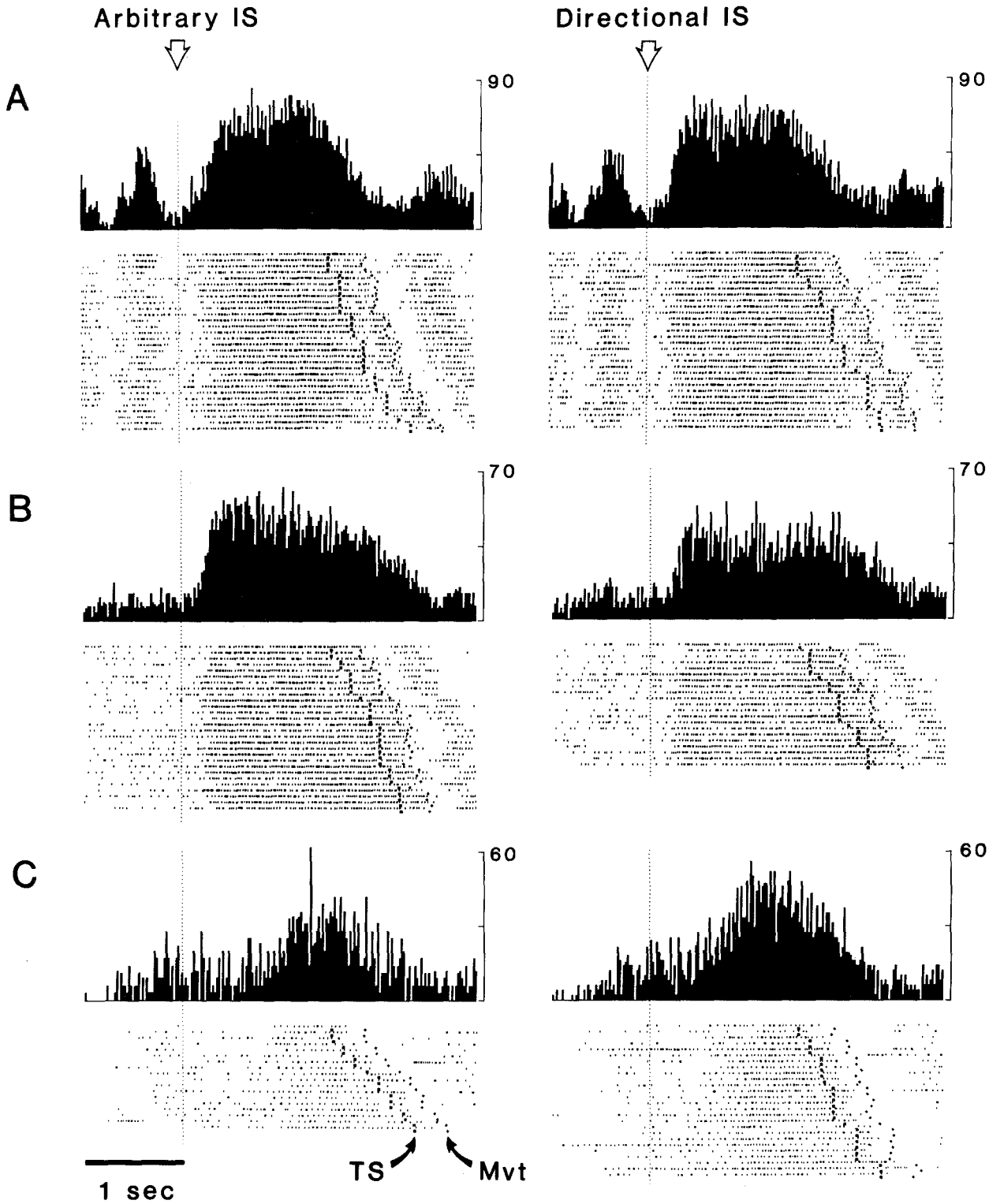

Fig. 3A–C. Three examples of set-related premotor cortex activity after arbitrary or directional instruction stimuli (open arrow). **A** A premotor cortex unit showing similar activity during the delay period of the two conditional tasks. **B** A unit showing higher activity when the IS was arbitrary than when it was directional. **C** A unit showing higher activity when the IS was directional than when it was arbitrary. **A** Shows activity for rightward trials, **B, C** for leftward trials. Raster displays and histograms are aligned on the presentation of either an arbitrary (left column) or a directional (right column) IS (open arrow). The first mark (a square) beneath each raster line indicates the onset of the trigger signal (TS) and the second mark (a cross) indicates the onset of movement (Mvt). Note that in this figure, and in Figs. 4, 6, and 7, the activity modulation to the left of the IS onset reflects the movement from a peripheral (left or right) to the central touch pad to initiate a new trial. The abscissa is scaled in impulses/s

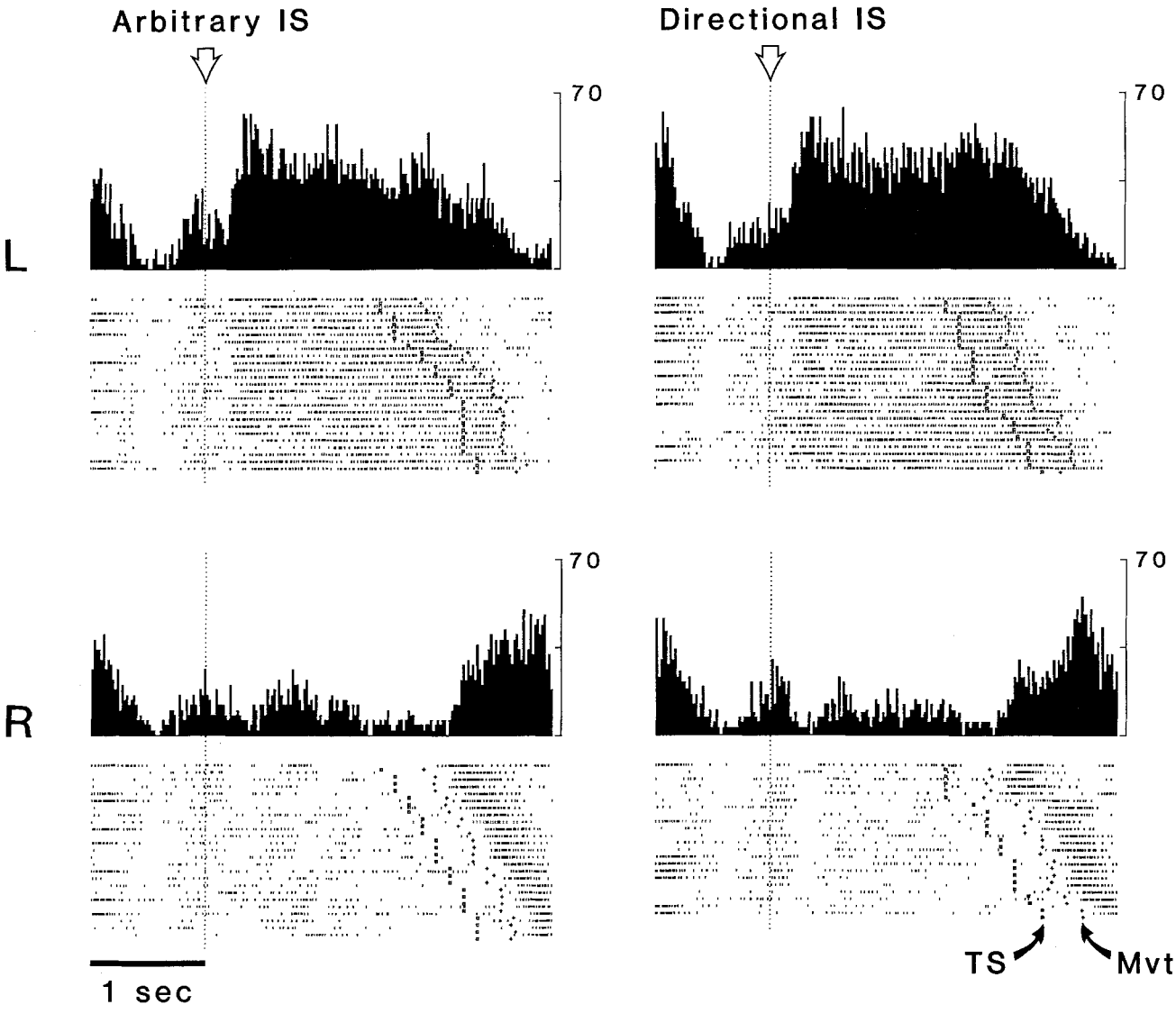

Fig. 4. Example of set-related premotor cortex activity showing selective activation for trials requiring one direction of forelimb movement. This neuron showed an increase in activity during the instructed delay period when the IS instructed the monkey to move its limb to the left (L), whereas it was relatively inactive during the delay period when the IS instructed a limb movement to the right (R). There was no statistically significant difference in the set-related activity that follows directional and arbitrary IS. Raster and histogram format as in Fig. 3

Results

Behavioral data

Reaction time (the time from the onset of the trigger signal to the onset of movement) and movement time (the time from the onset of movement to the acquisition of the target) were estimated for each direction of movement in each of the two tasks (Table 1). The time when the monkey's hand broke contact with the central touch pad was used as the movement onset time.

Both reaction time and movement time remained stable throughout the several weeks of recording from each animal (data not shown). There were small changes in reaction time and movement time during a day's recording session, as determined by an analysis of 28 selected sessions (12 for the first monkey), but similar changes occurred in the two tasks. The average reaction-time difference between the arbitrary and directional tasks in one direction was no more than 8 ms when the TS was nondirectional. Reaction and movement times of the first monkey were longer than those of the second monkey, but

Table 4. Numbers of set-related neurons displaying additional activity patterns grouped according to a comparison of set-related activity during the instructed delay period of the arbitrary vs. directional tasks

	A = D[a]	A > D	A < D	Total
Set[b] only	37	3	2	42
Set + Mvt	25	7	3	35
Set + Sig	9	0	0	9
Set + Ant	14	0	0	14
Set + Sig + Mvt	5	4	1	10
Set + Mvt + Ant	4	1	0	5
Set + Sig + Ant	0	1	0	1
Set + Mvt + Sig + Ant	2	0	0	2
Total	96	16	6	118

[a] Definition of A = D, A > D, and A < D as in footnotes a, b, and c, respectively, in Table 3

[b] *Abbreviations*: Set, set-related activity; Sig, signal-related activity; Mvt, movement-related activity (see Weinrich and Wise 1982); and Ant, anticipatory activity (see Mauritz and Wise 1986)

there were no statistically significant differences in these measures between the arbitrary and directional tasks for either animal or for either direction of limb movement. In both monkeys the reaction times for the leftward movements were significantly longer than those for the rightward movements. When the trigger signal was directional, reaction time increased (except for leftward movements in the arbitrary task) and movement time decreased (Table 1).

We examined whether the length of the delay period affected reaction time or movement time. The monkey could not predict precisely when the TS would be presented, but it may have been possible to predict the presentation of the TS toward the end of the longest delay periods. However, we did not find any consistent trend in reaction time or movement time dependent on the length of the delay period with the set of delay periods used in the present study (data not shown).

Muscle activity

The activity of a variety of muscles was sampled and analyzed (see Material and methods). Examples of EMG activity from the left forelimb of the second monkey during and before leftward and rightward movements are shown in Fig. 2. A number of muscles showed activity changes before or during forearm movements and following the presentation of the trigger signal. Muscles such as extensor carpi radialis, flexor carpi ulnaris, and biceps brachii increased activity before both directions of movement, while, in the initial premovement period, muscles such as deltoid revealed reciprocal activity (activation with one direction of movement and inhibition with the opposite direction of movement). The onset of change of EMG activity in the monkey's left forelimb started 30 ms to 130 ms prior to movement onset. EMG activity did not change significantly or consistently during the delay period, with the sole exception of the left rhomboid muscle in the second monkey, which showed decrementing activity after initiation of a trial until the onset of movement (Fig. 2). However, for that and all other muscles, the activity pattern of each muscle was at least qualitatively similar in the two conditional tasks.

Unit classification

A total of 403 task-related neurons were isolated in the premotor cortex. Units were classified into four nonexclusive categories on the basis of their discharge pattern: (1) *anticipatory neurons* became active before the presentation of the IS; (2) *signal-related neurons* showed transient activity modulation following the IS; (3) *set-related neurons* showed significant increases or decreases of sustained activity throughout most of the delay period; and (4) *movement-related* neurons changed activity following the trigger signal and preceding the movement onset (Weinrich and Wise 1982; Mauritz and Wise 1986). The distribution of premotor cortex neurons among the four major activity patterns is shown in Table 2. Seventy-five other task-related neurons, excluded from Table 2, showed activity that increased 1500 ms or more after IS onset (much later than set-related activity as previously defined), activity that increased or decreased after target acquisition, or activity that was too inconsistent to categorize among the four classifications listed above.

Comparison of set-related activity in the two conditional tasks

Out of 130 set-related neurons, 118 neurons showed significant increases in discharge rate during the instructed delay period, whereas 12 neurons showed significant decreases. The 118 excitatory set-related neurons were analyzed separately and in the most detail. Most set-related activity was virtually the same in the two conditional tasks, as shown in Table 3. Figure 3A shows set-related activity following arbitrary and directional IS that was not statistically significantly different in the period from 500 ms to 1500 ms after IS presentation (Mann-Whitney U-

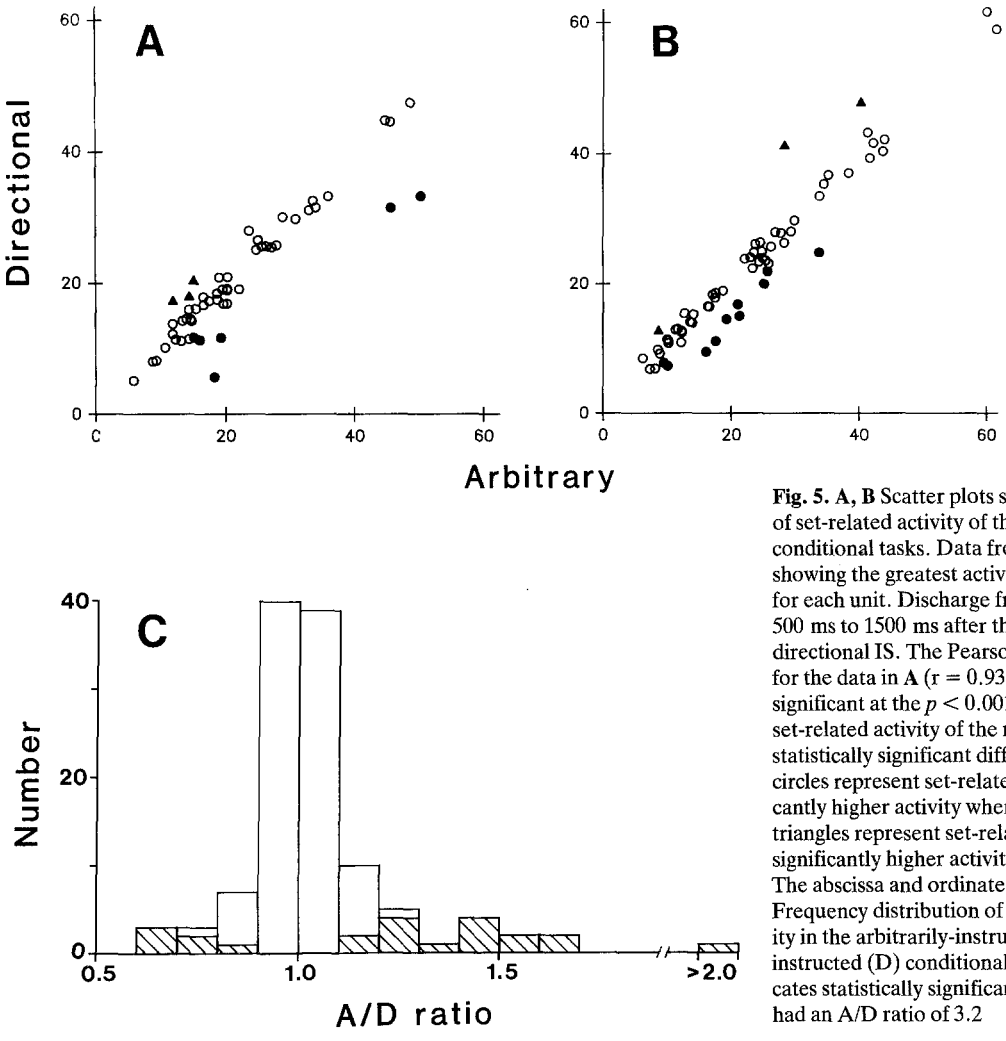

Fig. 5. A, B Scatter plots showing discharge frequency of set-related activity of the two monkeys in the two conditional tasks. Data from the movement direction showing the greatest activity modulation is displayed for each unit. Discharge frequency was averaged from 500 ms to 1500 ms after the onset of the arbitrary or directional IS. The Pearson's correlation coefficients for the data in **A** ($r = 0.93$) and **B** ($r = 0.97$) are both significant at the $p < 0.001$ level. Open circles represent set-related activity of the neurons that did not show a statistically significant difference in the two tasks. Filled circles represent set-related activity that showed significantly higher activity when the IS was arbitrary. Filled triangles represent set-related activity that showed significantly higher activity when the IS was directional. The abscissa and ordinate are scaled in impulses/s. **C** Frequency distribution of the ratio of set-related activity in the arbitrarily-instructed (A) to the directionally-instructed (D) conditional tasks (A/D). Hatching indicates statistically significant differences. The outlier had an A/D ratio of 3.2

Test at the 5% significance level). Ninety-six neurons showed no significant difference in set-related activity during the two tasks (and are termed A = D neurons). The total population of A = D neurons showed a change of 22.6 ± 11.5 impulses/s during the sampled delay period in the arbitrary task and the same 22.6 ± 11.3 impulses/s during the directional task. Figure 3B is an example of set-related activity that was higher when the IS was arbitrary than when it was directional (A > D neuron). The mean discharge rate changes during the sampled delay period for the 16 A > D neurons was 22.8 ± 11.1 impulses/s in the arbitrary task and 15.9 ± 8.2 impulses/s in the directional task. Set-related activity in Fig. 3C shows significantly higher activity when the IS was directional than when it was arbitrary (A < D neuron). The six A < D neurons had mean activity rate changes of 19.5 ± 10.6 impulses/s in the arbitrary task and 25.5 ± 11.9 impulses/s in the directional task during the sampled delay period.

All of the neurons shown in Fig. 3 had set-related activity before limb movements in either tested direction ("bidirectional" in Table 3), and for these neurons, the comparison of discharge rate in each conditional task was made for the direction showing the greatest set-related activity modulation. About one-third of the set-related neurons showed directional selectivity ("directional" in Table 3). This was defined as an increase in set-related discharge before movements in one direction and either a decrease or no change in activity before limb movements in the opposite direction. An example of directionally-selective set-related activity is shown in Fig. 4. Directionally selective cells showed a distribution of A = D, A > D, and A < D neurons similar to that of the bidirectional set-related cells (Table 3). There were 14 left-selective and 15 right-selective set-related neurons.

Although the neurons in Figs. 3 and 4 showed primarily set-related activity, a number of set-related

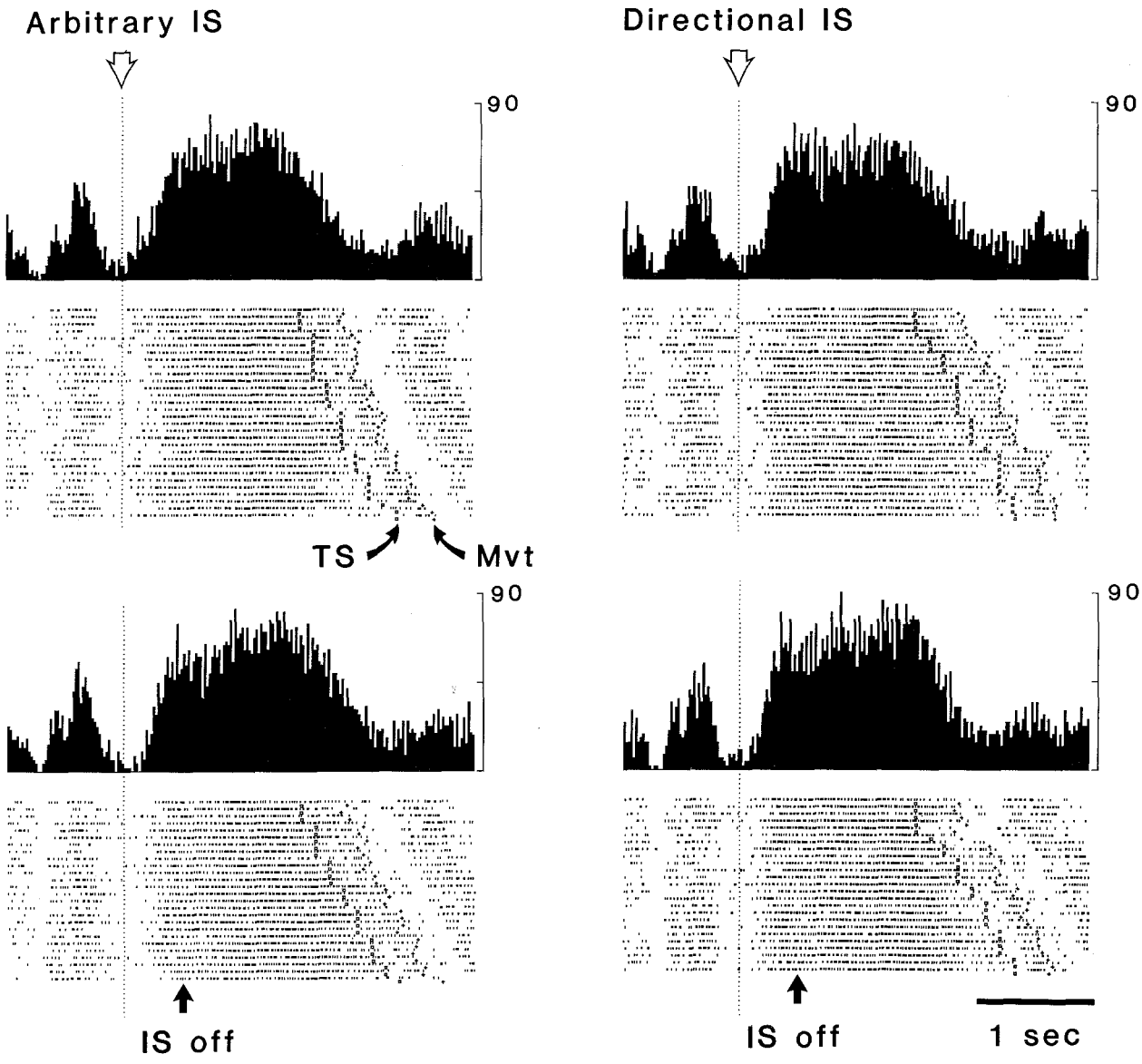

Fig. 6. Comparisons of set-related activity in IS-on (top row) and IS-off (bottom row) modes. This premotor cortex neuron showed similar activity when the IS remained on throughout the instructed delay period (IS-on mode) and when it was removed 0.5 s after presentation (IS-off mode). In addition, the activity in the IS-on and IS-off modes was similar regardless of whether the IS was arbitrary or directional

neurons also showed clear anticipatory, signal-related, or movement-related activity or some combination of those additional activity patterns. Table 4 shows that set-related neurons, when they also expressed other patterns of activity in relation to the task, were generally similar to the cells showing only set-related activity in their proportions of A = D, A > D, and A < D neurons, with the possible exception of neurons combining set-, signal-, and movement-related activity.

In order to compare the differences in set-related activity in the two conditional tasks, the mean discharge frequency modulation during the instructed delay period was plotted in Fig. 5A, B for each set-related neuron. The activity was measured from 500 ms to 1500 ms after the presentation of the IS. Examination of Fig. 5 shows that when differences existed between set-related activity in the two conditional tasks, these differences were generally small, even though statistically significant. The ratios of set-related activity in the arbitrary (A) to the directional (D) task ranged from 0.6 to 3.2, with 68% between 0.9 and 1.1. Eighty-one percent of set-related neurons had A/D ratios between 0.8 to 1.2

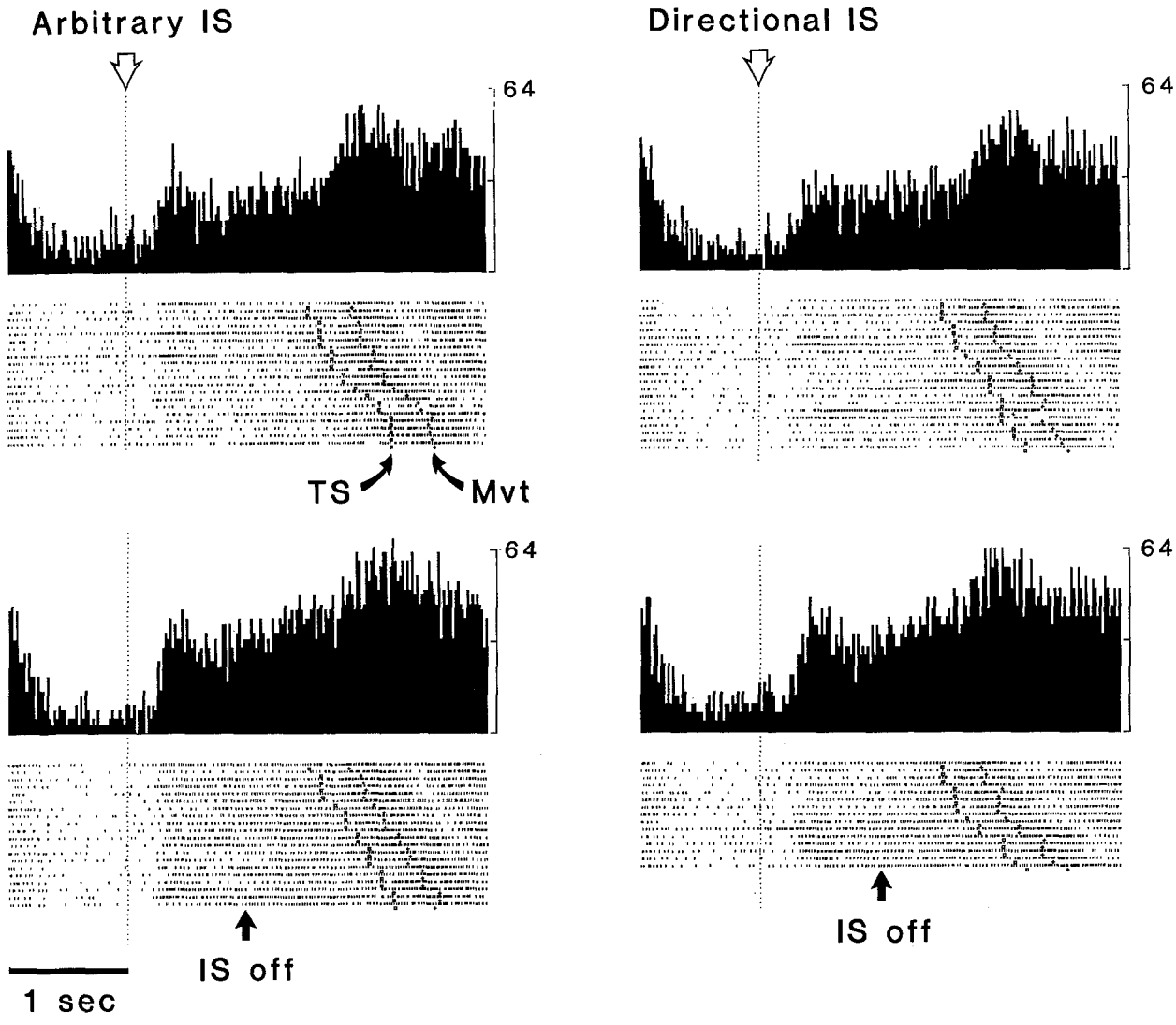

Fig. 7. Premotor cortex unit shown in the same format as Fig. 6. This cell showed statistically significantly higher activity when the IS was eliminated after a 1 s presentation (IS-off mode) than when it remained on throughout the trial (IS-on mode). It had a discharge rate of 34 ± 8 impulses/s in the IS-off mode and 25 ± 9 impulses/s in the IS-on mode (during the delay period from 500 ms to 1500 ms after IS presentation). This difference was significant at the $p < 0.01$ level (Mann-Whitney U-Test)

and had A/(D+A) ratios of 0.45 to 0.55. Figure 5C shows the distribution of A/D ratios.

Although we classified set-related activity as a basic cell discharge pattern, variations could be recognized qualitatively. For heuristic purposes, six visually distinguished variations are listed in Table 3. The first variation was by far the most common: the discharge rate changed soon after the presentation of the IS and its activity level remained relatively stable until just before the monkey initiated movement. This pattern of activity was observed in roughly 72% of 118 set-related premotor cortex neurons in the present sample (74% in the first monkey, 70% in the second). The second variation (6%) was incremental: the cell kept increasing its discharge rate continuously throughout the delay period. The third variation (8%) was decremental: the cell decreased its activity throughout the delay period. The fourth variation (8%), termed "early" in Table 3, was similar to the first one, but its discharge rate decreased significantly (though not to pre-IS levels) in the midst of the delay period. Most typically, set-related activity of this type decreased its discharge rate after the earliest possible occurrence of the TS (1520 ms after the onset of the IS). The fifth variation (6%) of set-related activity, termed "late" in Table 3, was, in a restricted sense, opposite to the fourth one: its activity increased shortly after

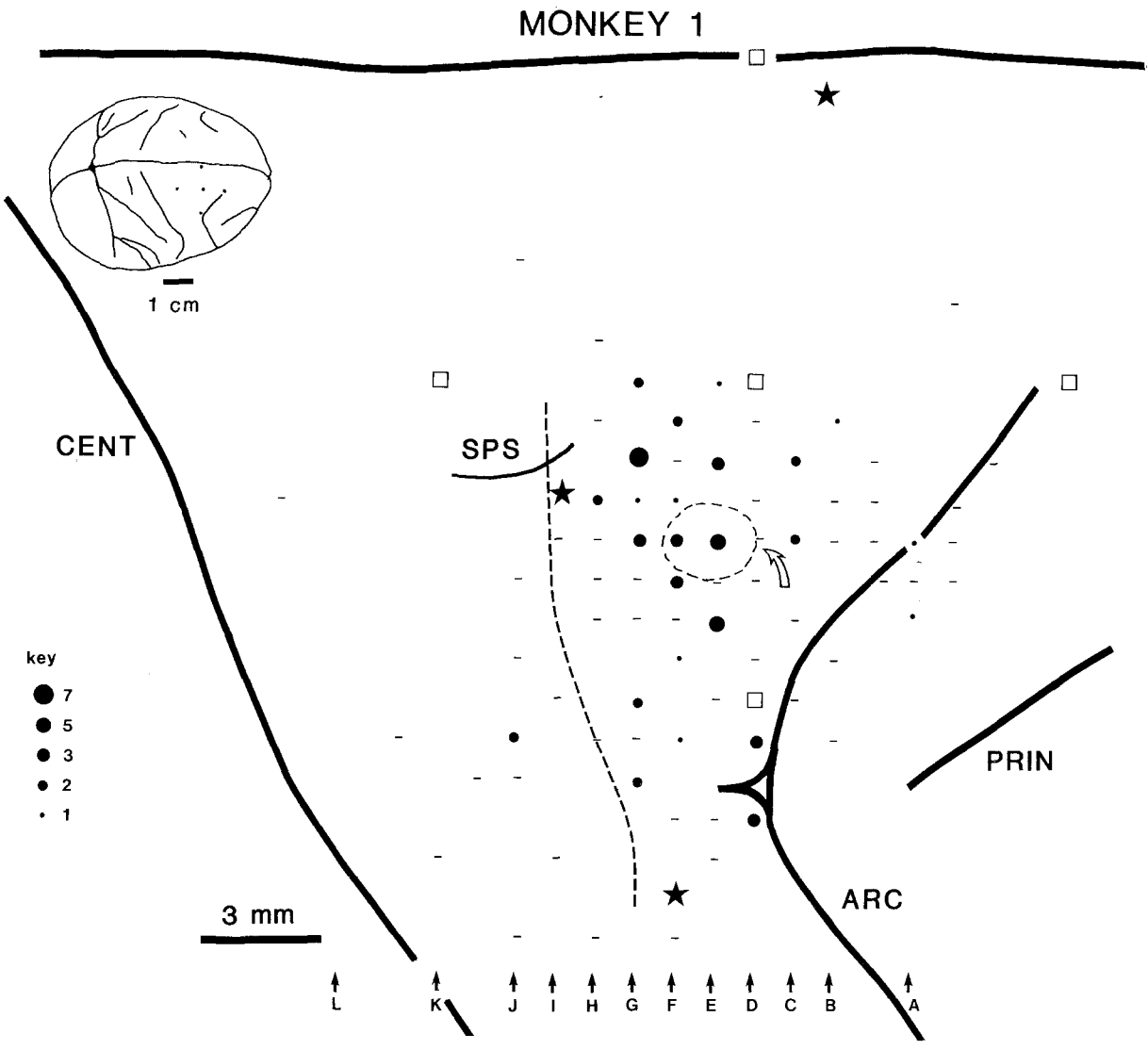

Fig. 8. Distribution of neurons with set-related activity in the right frontal lobe of the first monkey. Each filled circle represents the location of set-related cells, and its diameter is proportional to the number of such neurons in each electrode penetration (shown by the key). Dashes indicate the recording sites without set-related neurons. The inset at the upper left shows a surface view of the monkey's cerebral cortex. The open squares on the main diagram correspond to the dots on the inset and represent the location of pins inserted at known coordinates. The open arrow points to the site of gliosis (enclosed by the dashed line) associated with an injection into the cortex at known coordinates. The stars indicate the surface projections of the recovered electrolytic marking lesions. Solid arrows indicate the levels of sections so labelled in Fig. 9. The dashed, mainly vertical line indicates the cytoarchitectonically determined border between area 6 (rostral) and area 4 (caudal). Abbreviations for sulci: CENT, central sulcus; ARC, arcuate sulcus; SPS, superior precentral sulcus; PRIN, principal sulcus

the IS and then increased again in the midst of the delay period. In addition to this population of 118 cells, 17 neurons increased their discharge inconsistently (in time) and very late in the instructed delay period (termed "late, inconsistent" in Table 3). Although we did not include such cells in the set-related class (see Material and methods; Tables 2 and 4; and the top part of Table 3), it is not unreasonable to consider this discharge pattern a sixth variety of set-related discharge. None of these varieties show any appreciable proportion of cells that differ in the two conditional tasks, with the possible exception of the fourth one.

Comparison of set-related activity in task variations

Out of 130 set-related neurons, 42 neurons were examined to see if they showed a different discharge

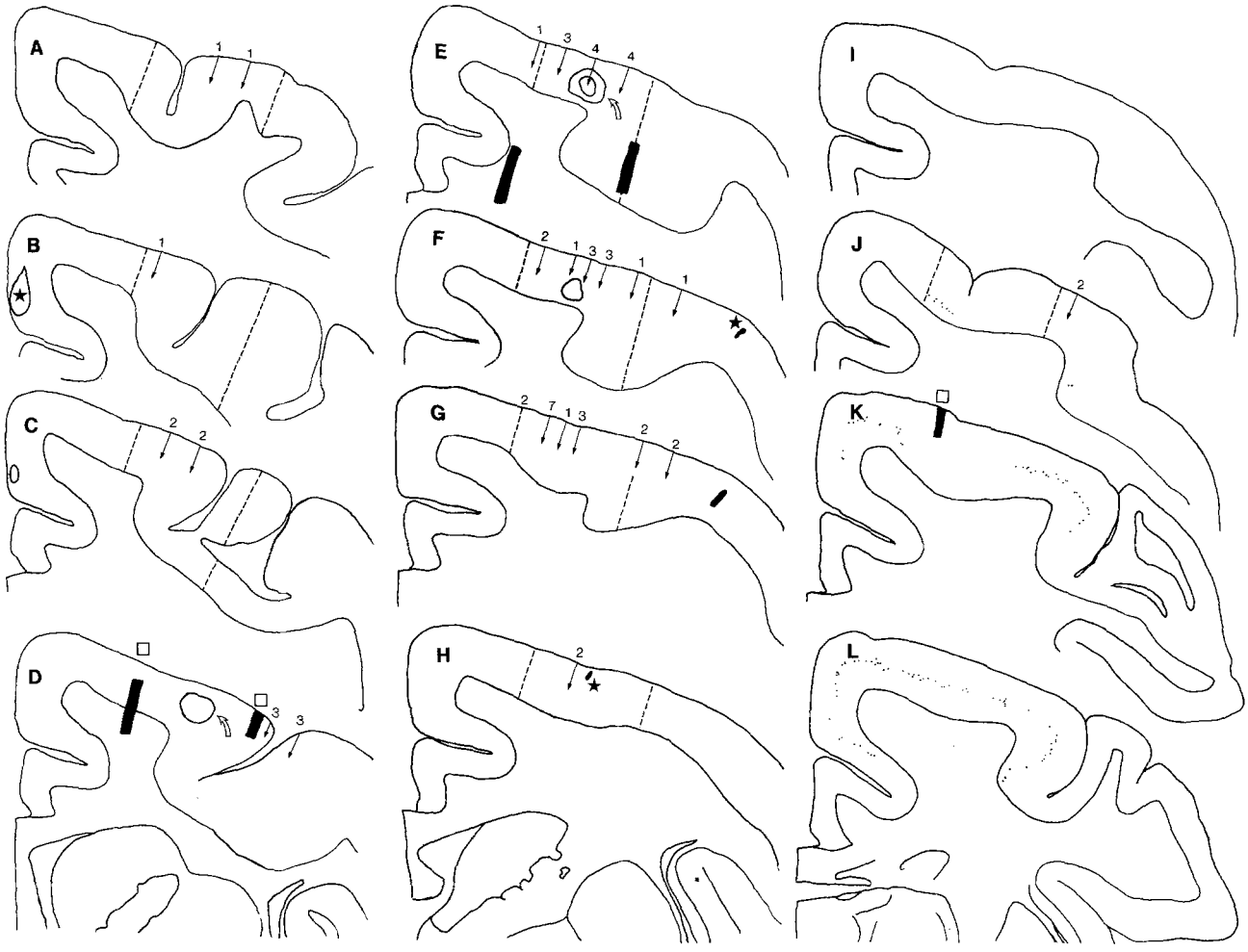

Fig. 9A–L. Frontal sections from the same brain illustrated in Fig. 8. Open squares mark in pin insertion sites, stars mark electrolytic lesions, and the open arrow marks the gliosis associated with an injection at known coordinates, as in Fig. 8. The black rectangles show the holes caused by pin insertions. The dashed lines indicate the reference system aligned on the center and lateral pin holes. Numbers at the pial surface indicate the number of set-related neurons isolated in the penetration marked by each arrow. Dots in sections **J**, **K**, and **L** show the location of the largest pyramidal cells. The depth of cells in each penetration was not analyzed. Each section is labelled to correspond to the upward pointing arrows in Fig. 8

rate or pattern when the IS was eliminated during the delay period (IS-off mode) or at the same time the TS was presented (IS-blank mode). Out of 42 cells thus examined, most (28) did not show any difference in activity in IS-off mode compared with that in IS-on mode (Fig. 6). Eleven cells showed higher activity throughout the delay period in the IS-off mode (Fig. 7). As a group, the activity for these 11 cells was 6 ± 4 impulses/s greater in IS-off than IS-on trials. Furthermore, three cells showed higher activity after the elimination of the IS in the IS-off mode, rather than, as for the 11 previously mentioned cells, throughout the instructed delay period. This activity increase lasted until movement onset. Set-related activity in the IS-blank mode compared with that in the IS-on mode showed a significant difference in only 1 of 7 cells tested (more activity in IS-blank mode). A comparison of set-related activity before directional vs. nondirectional trigger signals will be the subject of a subsequent report.

Localization

The approximate locations of set-related neurons are shown in Figs. 8 and 9 for the first monkey and in Fig. 10 for the second monkey. In the present study, the premotor cortex was defined cytoarchitectonically. The dorsolateral aspect of the frontal agranular cortex was divided into the precentral motor cortex,

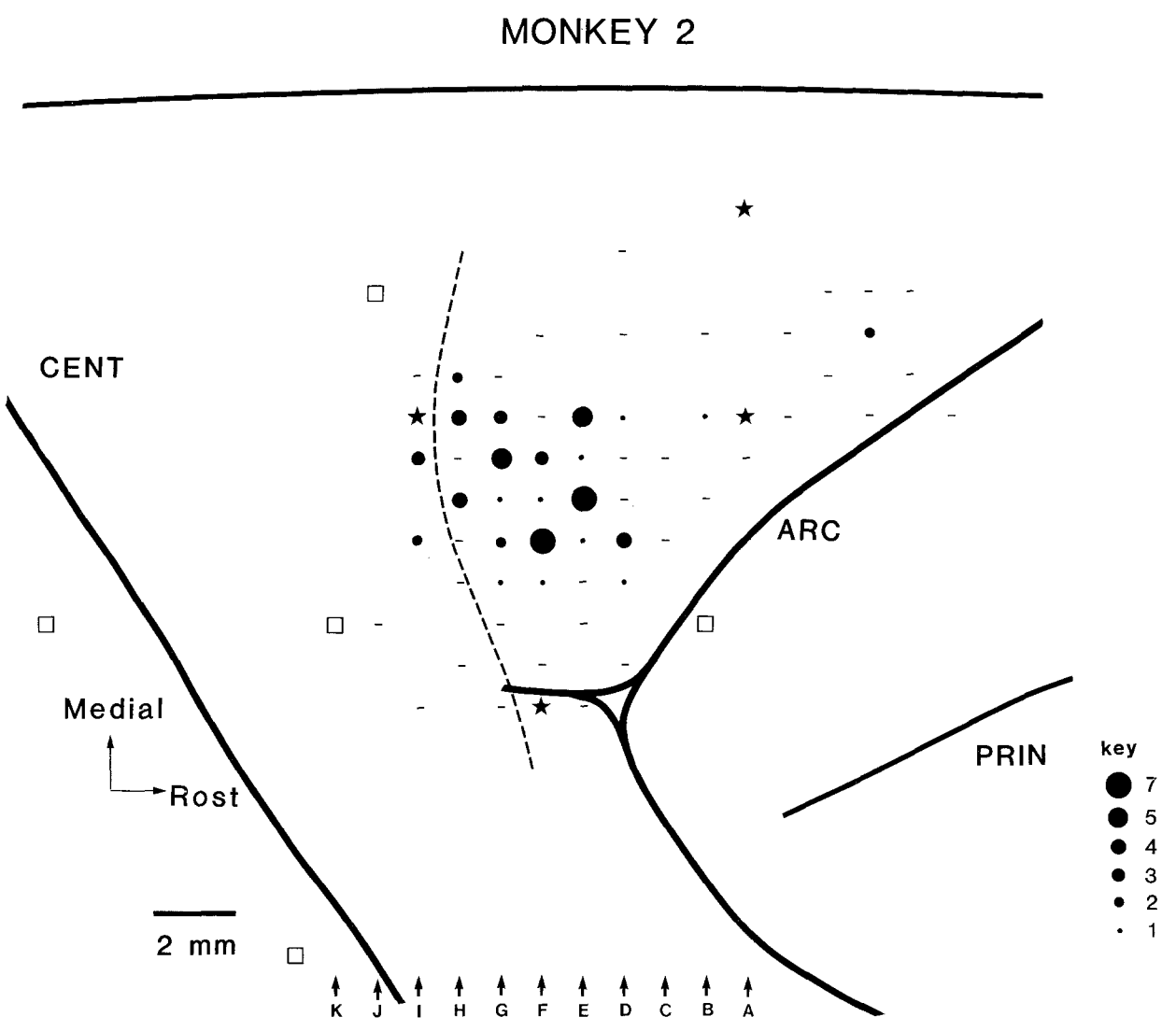

Fig. 10. Surface view of set-related neuron locations in the second monkey. Format as in Fig. 8. Abbreviations: CENT, central sulcus; ARC, arcuate sulcus; PRIN, principal sulcus; Rost, rostral

which approximately corresponds to the region that has the highest density of the largest layer V pyramidal cells (area 4), and the rostrally adjacent premotor cortex, approximately corresponding to the dorsolateral aspect of area 6. This delineation is based on previous studies (Weinrich and Wise 1982; Weinrich et al. 1984; Kurata and Tanji 1986; Sessle and Wiesendanger 1982) and arguments (Wise 1984a, 1985). The set-related cells studied in the present report were largely located within the part of the frontal agranular cortex characterized by the relative paucity of the largest layer V pyramidal cells. However, the possibility cannot be ruled out, especially for the second monkey (Fig. 10), that part of the neuronal population was located in the primary motor cortex. The location of A = D, A > D, and A < D set-related neurons were intermingled with no obvious concentration in any part of the premotor cortex (data not shown).

Discussion

Stimulus information processing vs. preparation for movement

Previous studies have supported the hypothesis that set-related activity in the premotor cortex reflects the preparation for upcoming limb movements (Wise et al. 1983; Weinrich et al. 1984; Godschalk et al. 1983;

Wise and Mauritz 1985). The observations in support of this view have been reviewed recently (Wise 1985b), but we emphasize two pertinent observations here. (1) When physically identical visuospatial cues under one condition instruct a monkey to make a limb movement of a given amplitude and direction and, under another condition, instruct the withholding of movement, the vast majority of premotor cortex set-related neurons are active exclusively or significantly more when the instruction is for a movement (Wise et al. 1983; Weinrich et al. 1984). And (2) when an instruction is changed during an instructed delay period, the set-related activity changes rapidly to reflect the new instruction. However, the set-related activity does not reflect the persistence of the visuospatial instruction stimulus (Wise and Mauritz 1985; Godschalk et al. 1985) or attention, gaze position, eye position, saccadic eye movements, arousal, reward contingencies, or motivation (Weinrich and Wise 1982; Weinrich et al. 1984; Godschalk et al. 1983, 1985). Although it is not possible to rule out with absolute certainty, a number of observations argue against the possibility that set-related activity is either a reflection of muscle activity or a motor command per se (Weinrich and Wise 1982; Weinrich et al. 1984; Wise and Mauritz 1985). The EMG data collected in the present study further supports that view, although one muscle, rhomboid, showed suppressed activity during the delay period in one monkey. For a discussion of the possible aspects of motor preparation in which the premotor cortex may be involved and its potential significance in behavioral adaptation, the reader is refered to previous publications (Evarts et al. 1984; Wise and Mauritz 1985).

The present study constitutes another test of the motor preparation hypothesis. Identical movements were instructed by very different visuospatial stimuli. If set-related premotor cortex activity reflects detailed processing of information concerning the sensory stimulus, then that activity would be expected to differ during directional and arbitrary tasks. In contrast, if the premotor cortex set-related activity reflects the preparation for specific limb movements, then that activity should not markedly differ in the two tasks. An examination of Fig. 5 shows that set-related activity, at least in the premotor cortex region explored here, does not markedly differ in the two tasks. Thus, the latter view is most supported by the present study.

Riehle and Requin (1985) have argued that premotor cortex cells reflect information processing. They presented monkeys (*M. fascicularis*) with instruction stimuli indicating either the direction or amplitude of the next limb movement, both direction and amplitude, or neither direction nor amplitude. The instruction was followed after a delay period by a trigger stimulus containing whatever information the instruction stimulus lacked about the direction and amplitude of the next movement. Riehle and Requin found premotor cortex neurons that were active during the delay period, but only when the monkey had been instructed about the direction of movement. The same cells were active just before movement and not during the delay period if the directional cue was given at the same time as the trigger stimulus (see also Riehle 1986). Riehle and Requin (1985) concluded that this activity reflected information processing. This conclusion would seem to conflict with ours, but we believe that the difference is only semantic. Their data appear to be consistent with the hypothesis that premotor cortex cells reflect the preparation for limb movement in a particular direction, whether that preparation occurs several seconds in advance of the movement (if the directional cue is delivered early enough) or just before the movement (if that information is delivered just before the movement). If this activity reflects "information processing" then it is information about the direction of movement, not the details of the sensory stimulus that conveys the instruction.

It is worth emphasizing that while we tentatively conclude that *set-related* activity reflects the preparation for specific limb action rather than processing sensory information, per se, this does not imply that the premotor cortex is unresponsive to sensory signals. There is evidence for premotor cortex responses to both visual (Rizzolatti et al. 1981b) and somatosensory (Wiesendanger et al. 1985; see also Rizzolatti et al. 1981a) stimuli, even when these stimuli may not be of any obvious behavioral significance to the animal.

Specification of the direction of limb movement or force

The recent work of Favilla et al. (1985) provides an additional perspective about the specification of directional motor behavior. Favilla et al. delivered separate cues about the direction and amplitude of force production, in this case for isometric limb contractions in humans. They found that the direction of isometric contraction and its amplitude appear to be programmed with different time courses during a premovement period. Direction was found to be programmed abruptly between 160 ms to 230 ms after an instruction. This compares well with the time course of set-related activity in the monkey premotor cortex (Weinrich et al. 1984; Wise and

Mauritz 1985), which typically appears robustly by about 130 ms to 140 ms after an instruction stimulus. By 160 ms after an instruction stimulus over half of the premotor cortex set-related cells have been "recruited", and by 230 ms (when direction is almost completely programmed according to Favilla et al.), more than 95% of set-related cells have shown a change in their discharge rate. Furthermore, the set-related activity we have observed fails to correlate with the amplitude of the upcoming limb movement or reaction time (Weinrich et al. 1984; Wise and Mauritz 1985), as if the premotor cortex cells reflect the specification of limb movement direction, independent of force or movement amplitude. It should be noted, however, that delay period activity of neurons in the primary motor cortex have been reported to correlate with reaction time (Kubota and Hamada 1979; Lecas et al. 1986).

What could be the advantage of specification of limb movement direction in the absence of amplitude programming? Perhaps the work of Riehle and Requin (1985) in macaques presents a clue: instructions giving prior information about the direction of movement resulted in much larger decreases in reaction time than those providing information about movement amplitude. It is possible that information about the direction of an upcoming movement causes a decrease in reaction time through modulation of responses to proprioceptive inputs. Bonnet (1984) and Bonnet and Requin (1984) found that directional instruction stimuli presented to humans lead to modulation of long-latency stretch reflexes. This modulation, only apparent when subjects are required to respond rapidly, might contribute to a decrease in reaction time through influences on joint stiffness or electromechanical delay. We propose that set-related premotor cortex neurons are part of a mechanism that decreases reaction time by preparing for subsequent limb movement direction, independent of movement amplitude or the force needed to generate it.

Relationship of neuronal activity to the effects of premotor cortex ablation

The hypothesis sketched in the previous paragraph is entirely consistent with the ideas about premotor cortex function developed on the basis of ablation studies (Halsband and Passingham 1982, 1985; Passingham 1985, 1986; Petrides 1982, 1985a, b, c, 1986). The preparation for limb movement direction can be viewed as an important aspect of the selection of a motor behavior on the basis of environmental context or, perhaps, the effect of such selection.

Behavioral studies, however, show that premotor cortex lesions cause very severe deficits in arbitrary conditional tasks (see Introduction), much more profound than a mere decrease in reaction speed. The contrast between these two ideas raises general questions about the comparison of neurophysiological data with those obtained from experimental ablation studies. There seems to be little question that premotor cortex neurons modulate their activity during behaviors in which the stimulus-response relation has a directional or visuospatial character. For example, during a direct reaching movement, for which the premotor cortex is not necessary (Moll and Kuypers 1977; Halsband and Passingham 1982), premotor cortex neurons profoundly modulate discharge rate (Weinrich and Wise 1982; Brinkman and Porter 1983). What we believe the behavioral data predict is that premotor cortex neurons should be at least as modulated in the arbitrary task as in the directional task. In accord with that prediction, Fig. 5 shows that even those differences in set-related activity during the two tasks that reached statistical significance were relatively small, and most of those favored the arbitrary task. Only six set-related neurons were more active when the instruction stimulus was directional, and these differences were usually small.

Reconciling the behavioral ablation effects and neurophysiological data is speculative, but not difficult: Perhaps the premotor cortex is necessary for learning or relearning certain conditional motor tasks, but not for performance of overlearned tasks in intact animals. After stable learning has occurred, the premotor cortex may then contribute to a mechanism that increases the speed of reaction.

Interpretational limitations

First, the present study is predicated on the existence of significant differences between the arbitrary and directional instruction stimuli: to the extent that they are similar our conclusion is weakened. Of course, the visuospatial stimuli presented are similar in several respects. Both the arbitrary and directional instructions have a certain minimal luminance, and are of the same size and approximately the same reflectance, and might be located in the same part of the visual field. While complex interactions between gaze and visual stimuli could complicate the interpretation of cell activity, there is no reason to think that set-related activity codes the stimulus features noted above (Rizzolatti et al. 1981; Weinrich and Wise 1982; Gentilucci et al. 1983; Weinrich et al. 1984; Wise and Mauritz 1985; see also Godschalk et al.

1985). Second, it is possible that the so-called directional instruction is treated arbitrarily by the monkey, or that, since the task was highly overlearned, the instruction stimuli lost their arbitrary character. And third, it remains possible that we failed to see differences between set-related activity in the directional and arbitrary conditional tasks because of a regional sampling bias. Matelli et al. (1985, 1986) have suggested the existence of two functionally distinct parts of the premotor cortex, each with a forelimb representation: one inferior and the other superior to the spur of the arcuate sulcus. If this distinction is valid, then our conclusions apply mainly to the superior representation, whereas those of Godschalk et al. (1985) apply primarily to the inferior one. Since set-related activity is observed throughout wide areas of the cerebral cortex (Evarts et al. 1984; Lecas et al. 1986), it is possible that neuronal activity elsewhere, perhaps in other parts of the frontal cortex, would show clearer specificity for arbitrary or directional conditional tasks.

Conclusion

The present study supports the hypothesis that set-related premotor cortex activity reflects the preparation for limb movements and is consistent with behavioral findings that indicate a premotor cortex contribution to the mechanisms underlying behavioral flexibility (see Evarts et al. 1984).

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